SUPPLEMENTARY INFORMATION

Supplementary Note 1 | Reproducibility of Saturation Genome Editing Experiments

The correlations between replicates for each of the experiments suggest that while this technique reproducibly measures effects of many programmed genome edits in parallel, there are also non-trivial sources of noise. Here, we elaborate further on why this is and emphasize the importance of performing biological replicates.

Several lines of evidence suggest that the noise observed relates to the fact that modest editing efficiencies lead to relatively few cells in each experiment harboring each specific edit. In the *BRCA1* hexamer experiment and the *DBR1* experiment, we observe a bimodal distribution of gDNA read counts (**Extended Data Figs 1a, 9b**). This is consistent with a bottleneck resulting in not all HDR library edits being present in post-editing gDNA. If some edits are not present at all, it follows that some edits are only made once or a few times, and are represented by very few cells when measurements are made, possibly as few as one.

Consistent with lowly sampled edits being more prone to noise, we observe that hexamers that are more highly represented in gDNA counts are more reproducible. For example, whereas R = 0.659 between two biological replicates overall, hexamers falling into the top third with respect to gDNA count correlated much more highly (R = 0.857). Furthermore, considering the two *BRCA1* experiments, we note that because there were far fewer possible SNVs (n = 234; experiment in Figure 2) than hexamer substitutions (n = 4,095; experiment in Figure 1), each individual edit is expected to be created independently many more times during editing, given a generally similar experimental setup with respect to number of cells, functional assay, etc. We believe this is what gives rise to the higher reproducibility of the SNV effect sizes as compared with the hexamer enrichment scores (*i.e.* Figure 2a vs. Figure 1b).

Whether the noise represents biological variability (for instance, two cells with the same edit producing transcripts at different rates) or technical variability (stochastic effects inherent to sample prep) we reasoned that by pooling or averaging replicates, we effectively increase the number of successfully edited cells sampled, and therefore reduce noise attributable to low sampling. Consistent with this, pooling read counts from D3 replicates in the *BRCA1* hexamer experiment improved correlation between biological replicates.

For the *DBR1* experiment, the overall reproducibility of D11 enrichment scores is reasonable (R = 0.752; **Extended Data Fig. 10c**). In each biological replicate, we observe a bimodal distribution of enrichment scores (*i.e.* corresponding to tolerated vs. deleterious). While there are a handful of unexpected observations, *e.g.* nonsense mutations that are tolerated in one replicate, nearly all of the unexpected observations did not replicate. While two synonymous changes score as reproducibly deleterious, there are no nonsense or frameshift mutations that are reproducibly tolerated.

This experiment, subject to bottlenecking at the editing step, generates clonal populations possibly expanded from a single edited cell. Falsely tolerated edits (i.e. nonsense mutations not selected against) in a given replicate could be explained by Hap1 cells' reversion to diploidy

prior to editing occurring, a noted disclaimer from Haplogen (the cell line's source). Falsely deleterious edits in a given replicate could be observed due to off-target CRISPR cutting in other essential regions, or random dropout when half the sample is split on D5.

These findings suggest that while the technique is sensitive enough to measure effects from very few edited cells, noise associated with sampling such small populations mandates the necessity of replicating data sets to improve confidence in the measurements associated with individual genome edits. Our data also suggest that future experiments will benefit from increased reproducibility achievable by a) transfecting and analyzing a higher number of cells, b) limiting complexity of HDR libraries, or c) improving HDR efficiency to allow for sampling of more edited cells.

Supplementary Note 2 | Potential Applications of Saturation Genome Editing

In the experiments presented, we took advantage of strategies that directly linked genotype to phenotype to assay pools of multiplex HDR-derived variants in parallel. As we show, targeted RNA and DNA sequencing of the edits themselves via selective PCR are well suited to catalog variants' effects on splicing and cellular fitness, respectively. However, we believe that with relatively simple adaptations of the method, complex pools of genome edits can be subjected to many additional assays that measure diverse aspects of biology.

First, the approach illustrated in the *BRCA1* experiments is broadly applicable to study how genomic variation within virtually any transcribed element affects its own RNA abundance. Specifically, this approach could readily be adopted to study how other transcribed elements contribute to expression levels (*e.g.* the influence of 5'- and 3' UTRs sequence on RNA stability, etc.). In this context, we also note that enhancers are transcribed at low levels (eRNA), suggesting an approach for studying enhancer activity, as well.

Additionally, assays such as targeted ChIP-Seq could be performed to characterize how libraries of genomic edits affect epigenetic states in coding or non-coding regions. By taking large quantities of DNA from expanded populations of edited cells and functionally separating edits based on biochemical interactions (i.e. transcription factor binding, associated histone modification, nucleosome positioning, etc.), genotype-phenotype associations would be preserved.

Apart from the molecular assays described above, the *DBR1* experiment is just one example of a cell-based assay that can be read out with high-throughput sequencing. In addition to essentiality (in haploid cells or diploid cells made functionally haploid through previous gene disruption), gain-of-function (such as drug resistance or growth gain), haploid insufficiency and dominant negative effects could be measured with appropriate selection assays. In fact, any well-customized assay that allows functionally-based separation of cell populations (e.g., with FACS) is amenable to downstream sequencing of edited populations of assayed cells as a readout. For instance, reporter cell lines engineered to express fluorescently tagged genes of interest could be used to assay multiplex HDR-edited transcription factors or enhancers.

Given the relative ease of targeted nuclease production and mutagenesis library cloning, the method that we present is readily scalable. Exons could be tiled to functionally assess each coding SNV across entire genes. In summary, although further optimization is required, we predict that with continued improvement and adaptation to a large variety of assays, this method will be a valuable approach for determining functional effects of large numbers of programmed genomic mutations in many biological contexts.

Type of file: figure
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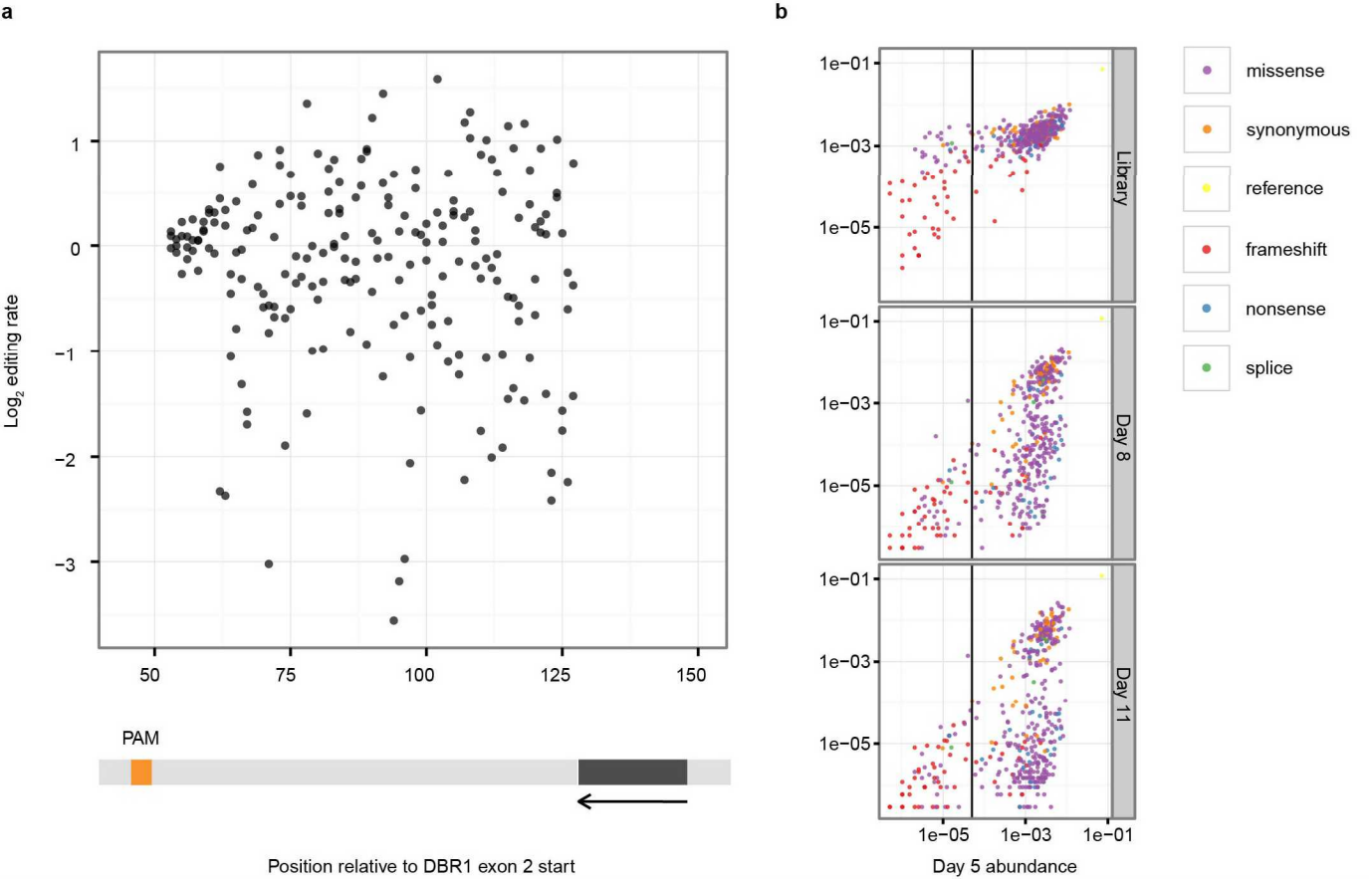
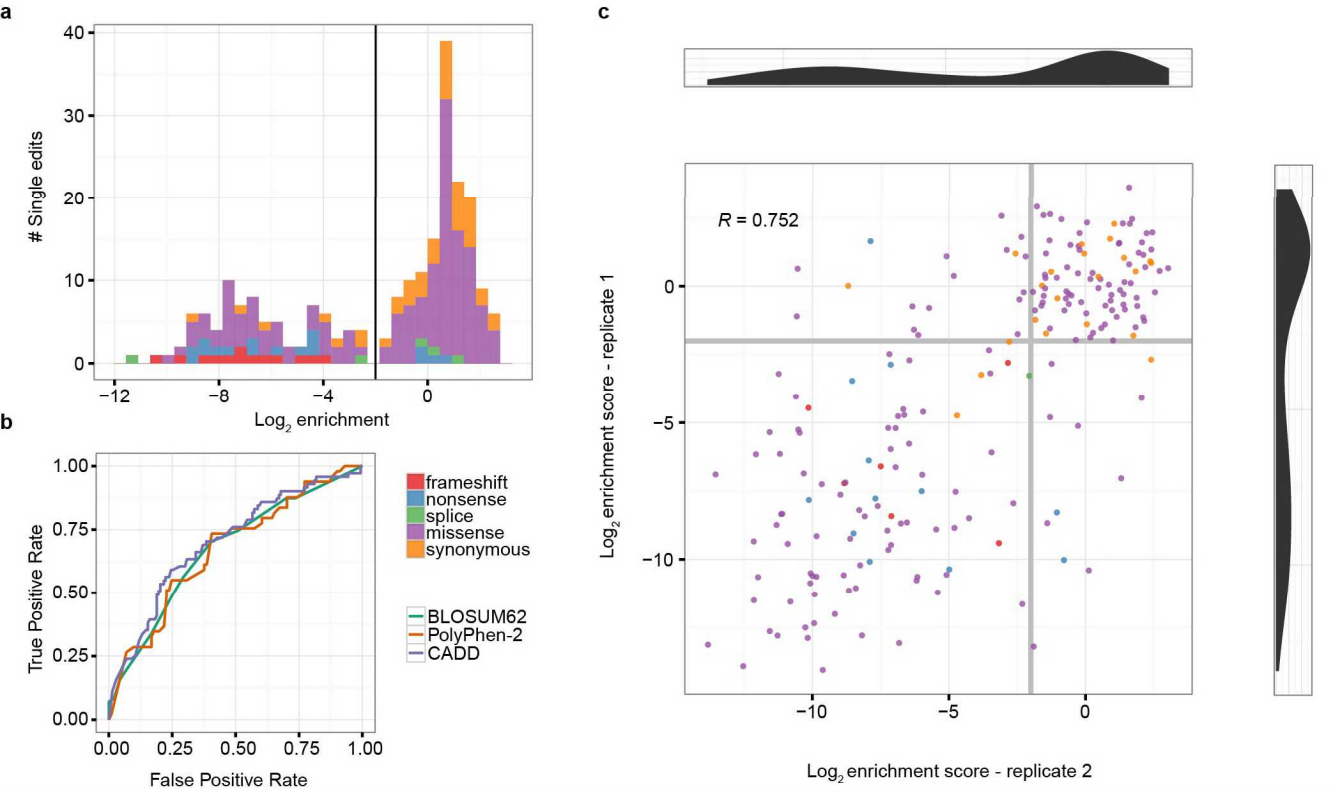
Filename: supp_info_10.jpg


a
b
Log2 editing rate
1
0
−1
−2
−3
50
75
100
125
150
PAM
Position relative to DBR1 exon 2 start
1e−01
1e−03
1e−05
Library
Day 8
Day 11
Day 5 abundance
missense
synonymous
reference
frameshift
nonsense
splice

Type of file: figure
Label: extdata11
Filename: supp_info_11.jpg


a
Single edits
Log2 enrichment
b
True Positive Rate
False Positive Rate
frameshift
nonsense
splice
missense
synonymous
BLOSUM62
PolyPhen-2
CADD
c
R = 0.752
Log2 enrichment score - replicate 1
Log2 enrichment score - replicate 2

Type of file: figure
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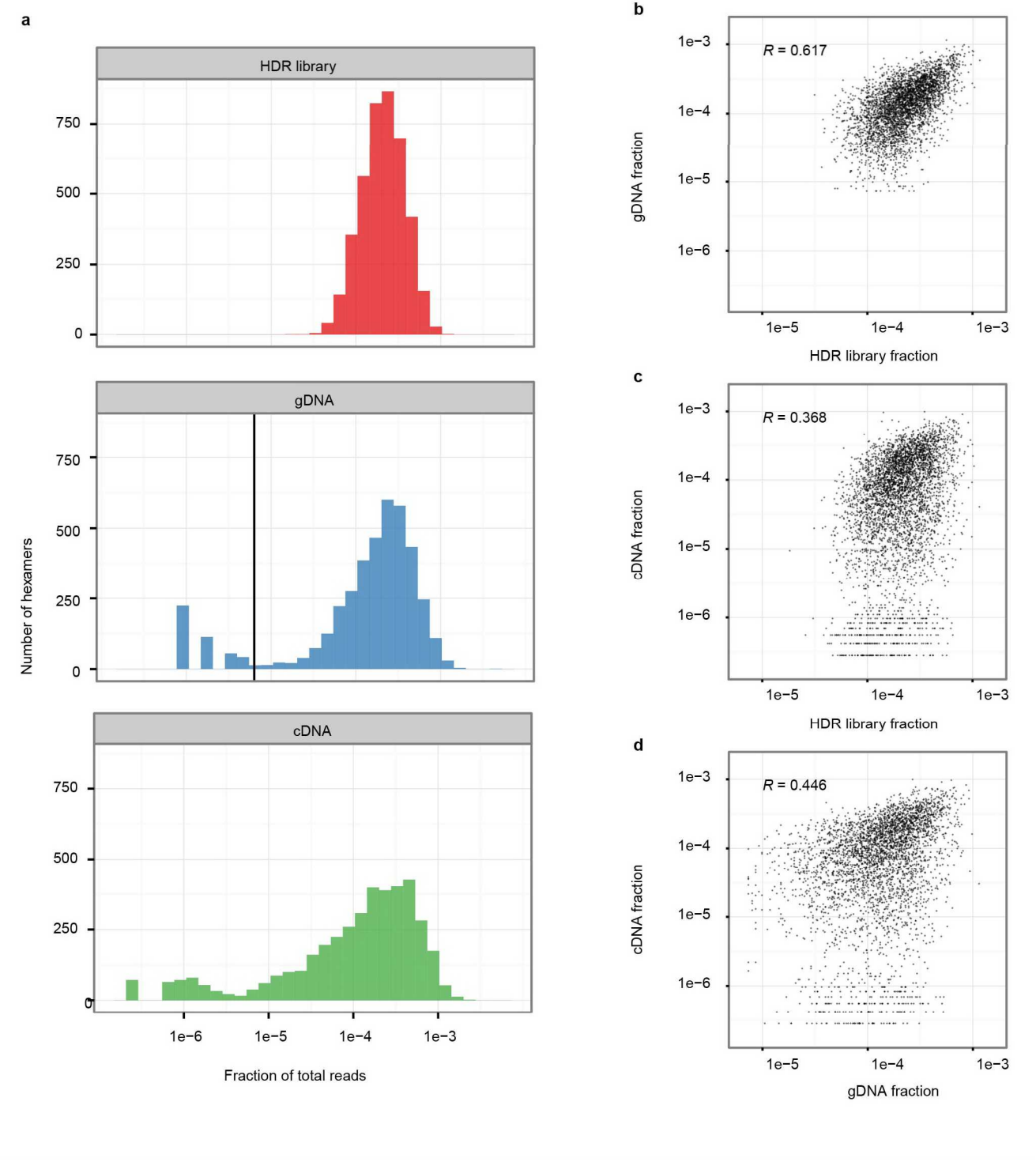
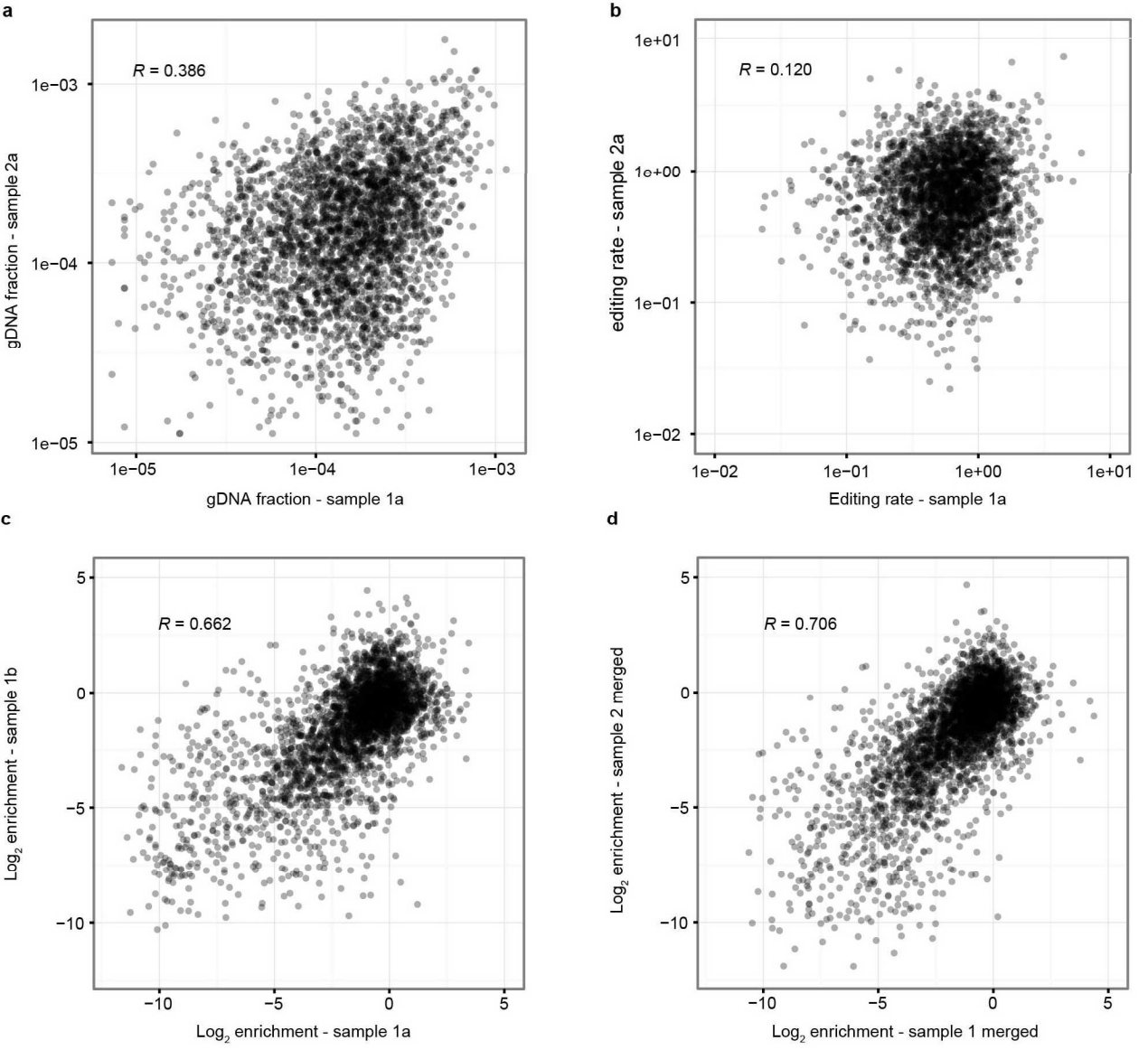
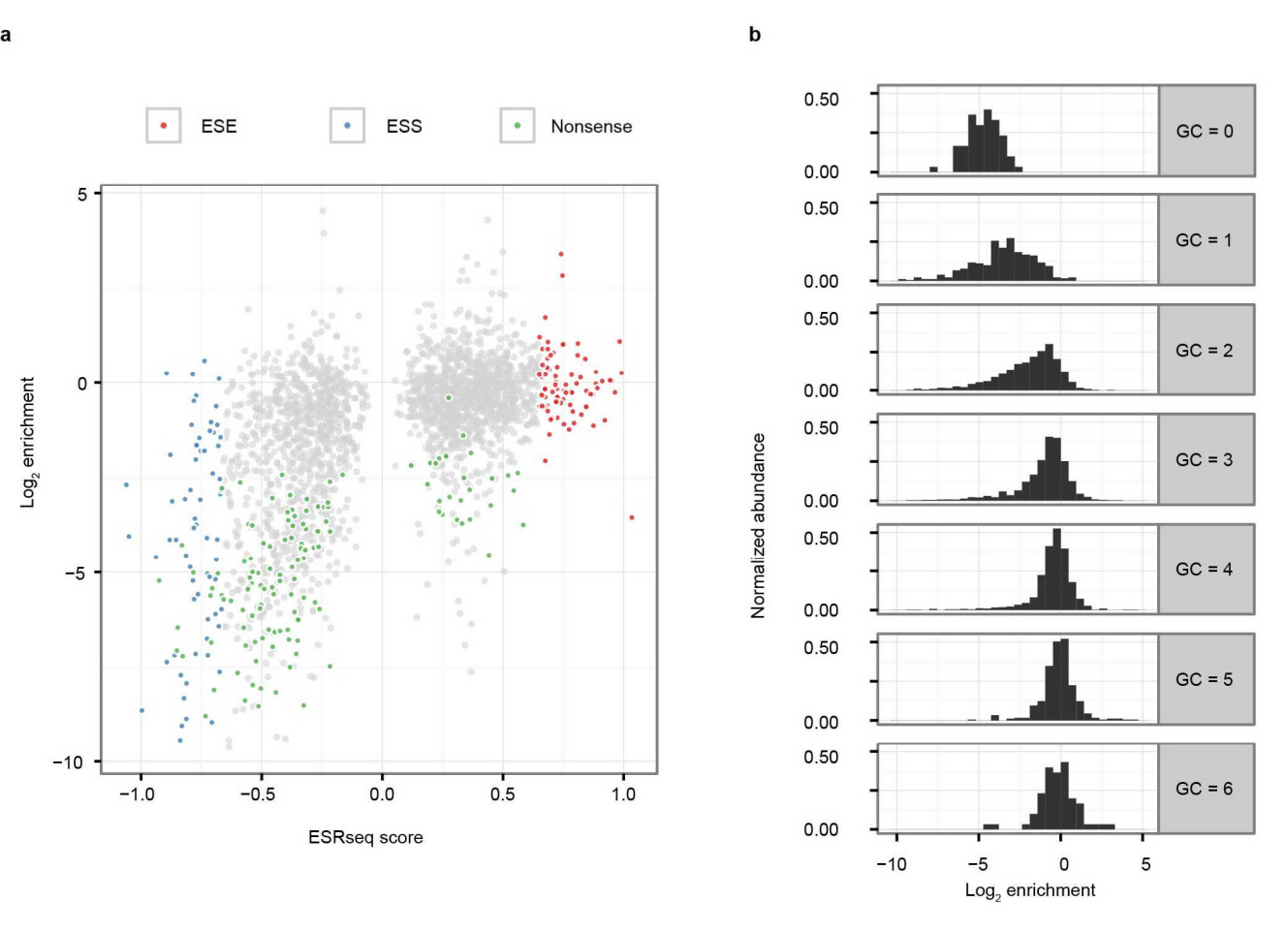
Filename: supp_info_2.jpg


a
HDR library
gDNA
cDNA
Number of hexamers
750
500
250
0
1e−6
1e−5
1e−4
1e−3
Fraction of total reads
b
R = 0.617
gDNA fraction
HDR library fraction
c
R = 0.368
cDNA fraction
HDR library fraction
d
R = 0.446
cDNA fraction
gDNA fraction

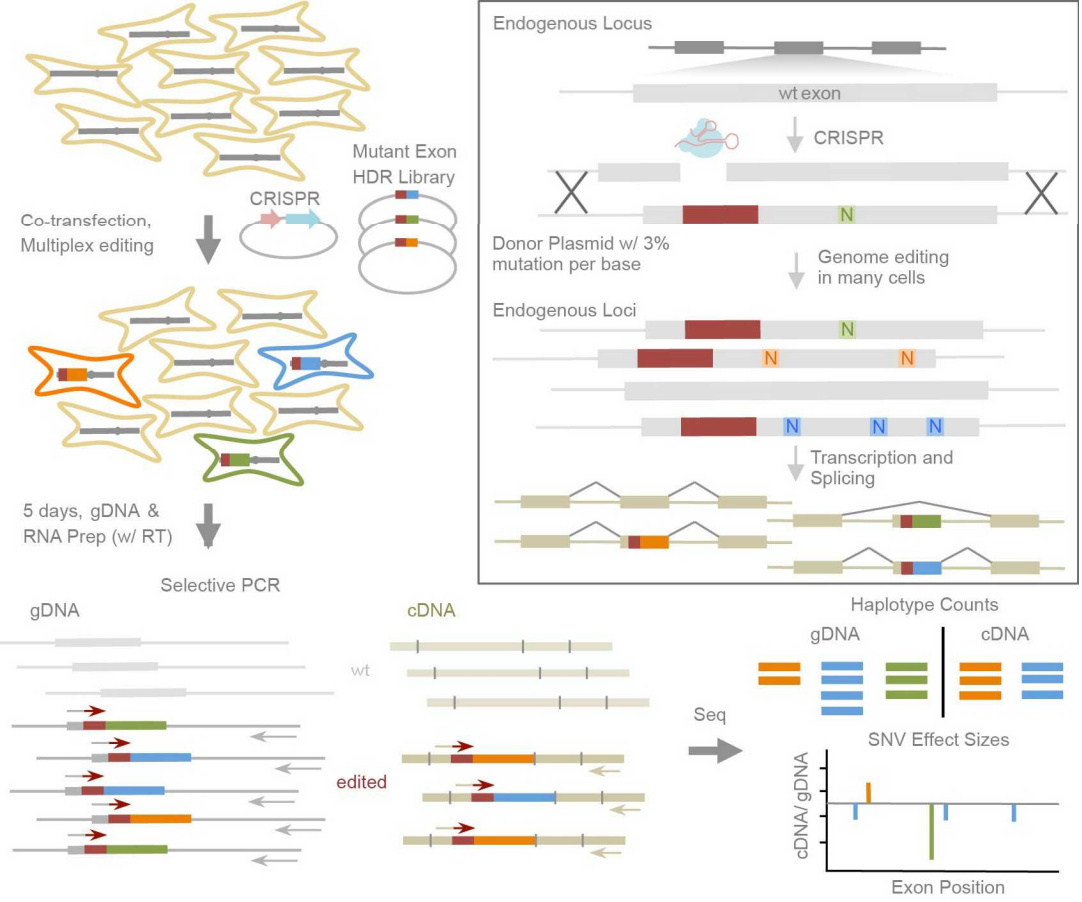
Type of file: figure
Label: extdata3
Filename: supp_info_3.jpg


a
R = 0.386
1e−03
1e−04
1e−05
gDNA fraction - sample 2a
1e−05
1e−04
1e−03
gDNA fraction - sample 1a
b
R = 0.120
1e+01
1e+00
1e−01
1e−02
editing rate - sample 2a
1e−02
1e−01
1e+00
1e+01
Editing rate - sample 1a
c
R = 0.662
5
0
−5
−10
Log2 enrichment - sample 1b
−10
−5
0
5
Log2 enrichment - sample 1a
d
R = 0.706
5
0
−5
−10
Log2 enrichment - sample 2 merged
−10
−5
0
5
Log2 enrichment - sample 1 merged

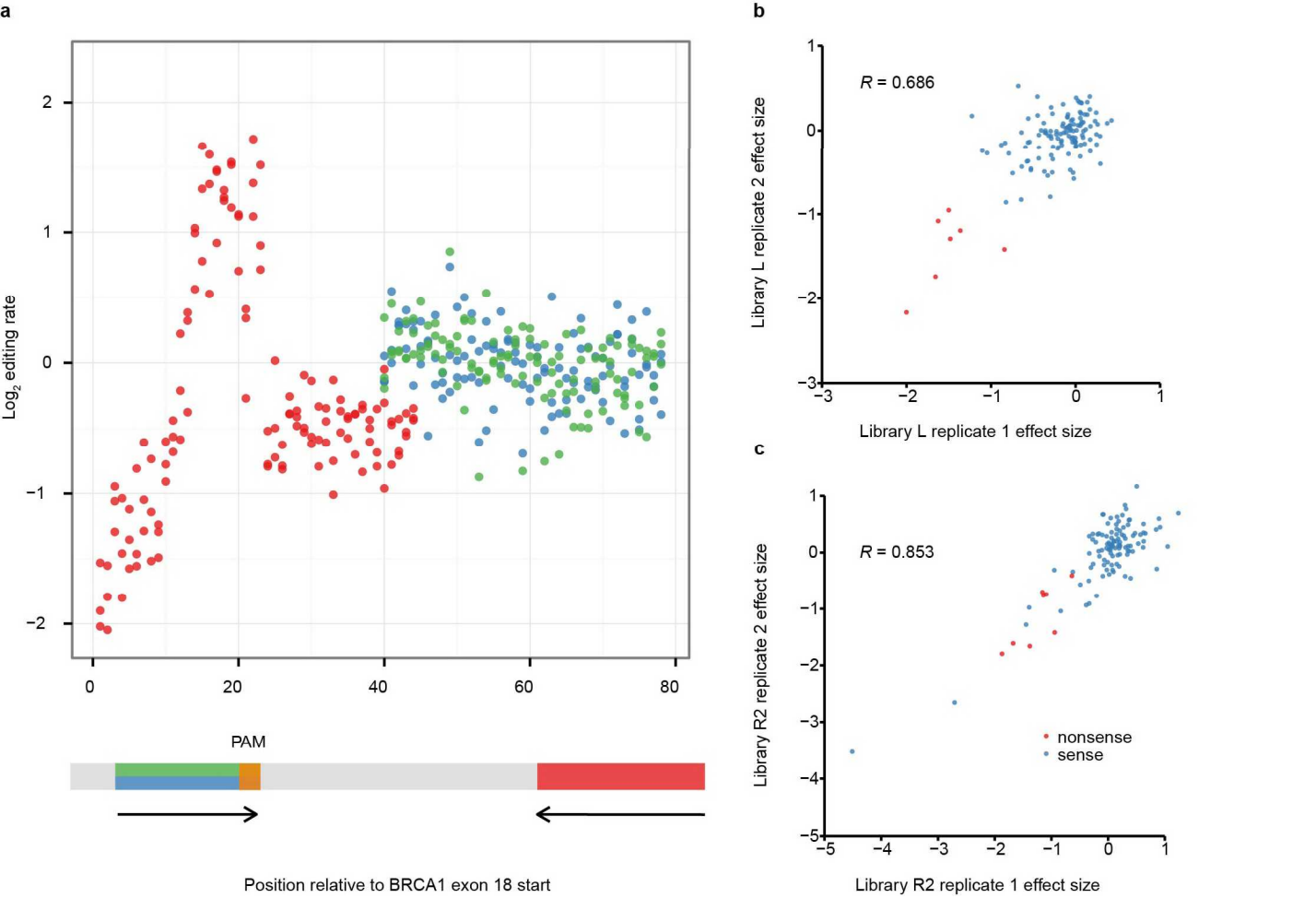
Type of file: figure
Label: extdata4
Filename: supp_info_4.jpg


a
ESE
ESS
Nonsense
5
0
−5
−10
Log2 enrichment
−1.0
−0.5
0.0
0.5
1.0
ESRseq score
b
Normalized abundance
0.50
0.00
GC = 0
GC = 1
GC = 2
GC = 3
GC = 4
GC = 5
GC = 6
−10
−5
0
5
Log2 enrichment

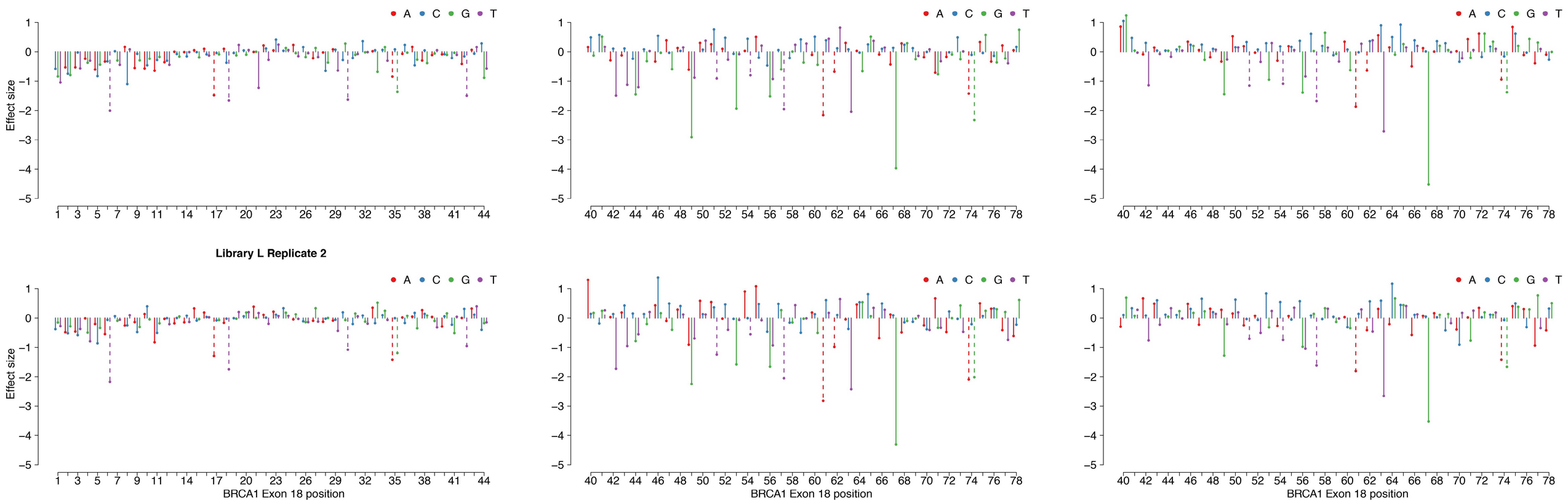
Type of file: figure
Label: extdata5
Filename: supp_info_5.jpg


Endogenous Locus
wt exon
CRISPR
N
Donor Plasmid w/ 3%
mutation per base
Genome editing
in many cells
Endogenous Loci
N
N
N
N
N
Transcription and
Splicing
Mutant Exon
HDR Library
CRISPR
Co-transfection,
Multiplex editing
5 days, gDNA &
RNA Prep (w/ RT)
Selective PCR
gDNA
cDNA
wt
edited
Seq
Haplotype Counts
gDNA
cDNA
SNV Effect Sizes
cDNA/ gDNA
Exon Position

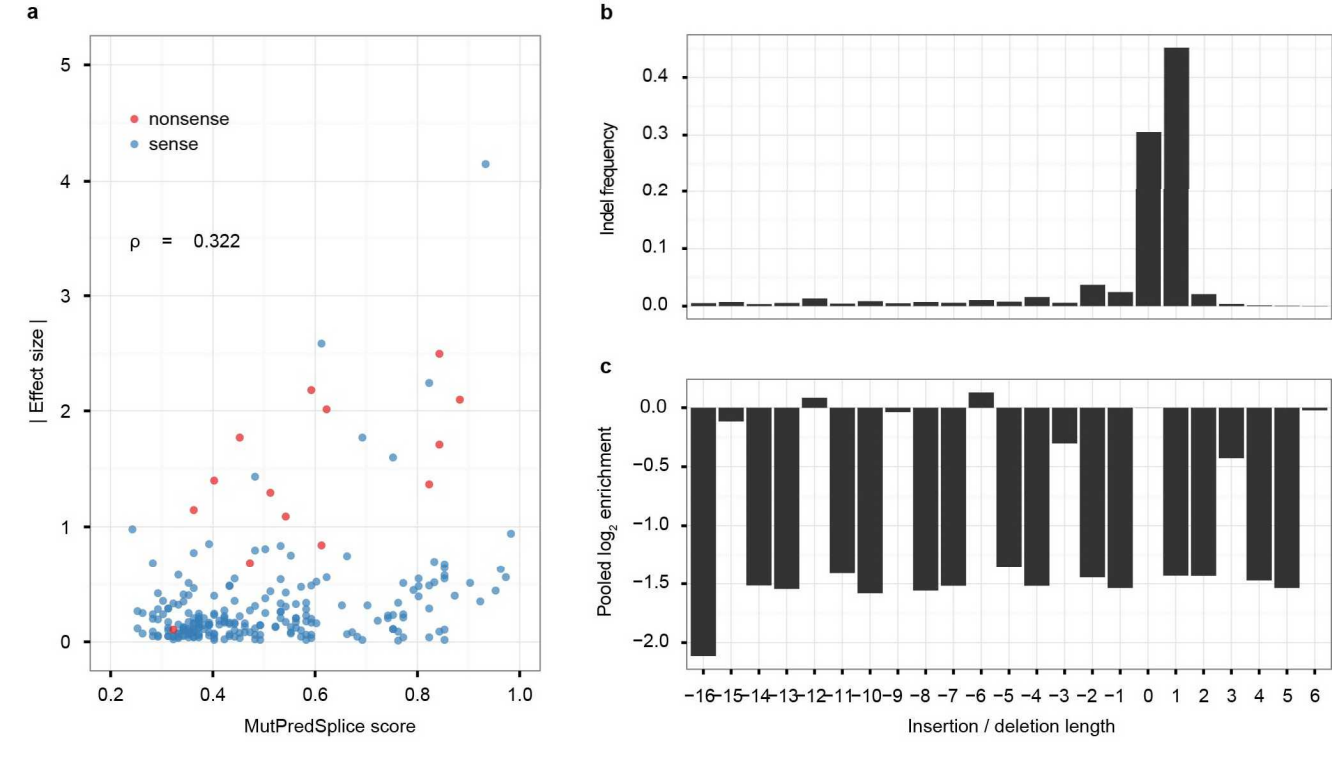
Type of file: figure
Label: extdata6
Filename: supp_info_6.jpg


a
b
c
Log2 editing rate
2
1
0
−1
−2
0
20
40
60
80
PAM
Position relative to BRCA1 exon 18 start
R = 0.686
Library L replicate 2 effect size
Library L replicate 1 effect size
−3
−2
−1
0
1
R = 0.853
Library R2 replicate 2 effect size
Library R2 replicate 1 effect size
−5
−4
−3
−2
−1
0
1
nonsense
sense

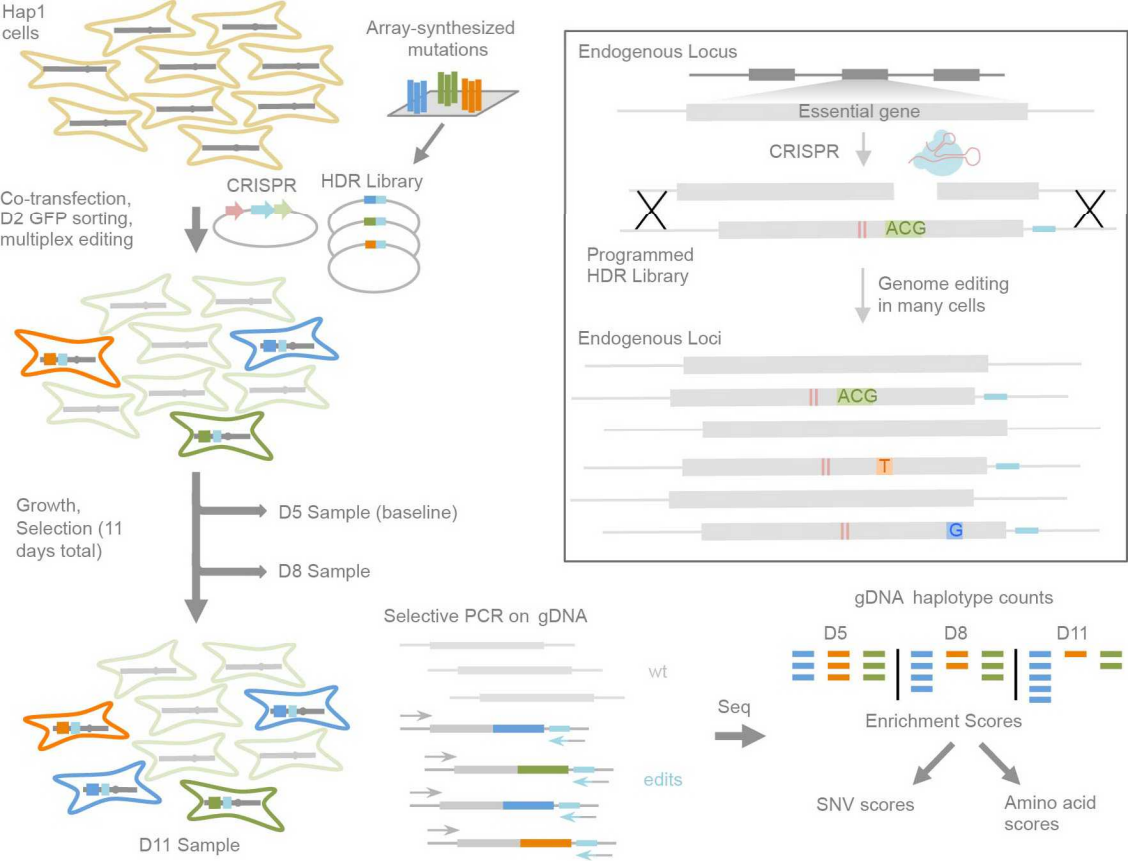
Type of file: figure
Label: extdata7
Filename: supp_info_7.jpg


Library L Replicate 2
A C G T
Effect size
1
0
−1
−2
−3
−4
−5
1 3 5 7 9 11 14 17 20 23 26 29 32 35 38 41 44
40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78
BRCA1 Exon 18 position

Type of file: figure
Label: extdata8
Filename: supp_info_8.jpg


a
b
c
nonsense
sense
ρ = 0.322
| Effect size |
MutPredSplice score
Indel frequency
Pooled log2 enrichment
Insertion / deletion length

Type of file: figure
Label: extdata9
Filename: supp_info_9.jpg


Hap1 cells
Array-synthesized mutations
CRISPR
HDR Library
Co-transfection, D2 GFP sorting, multiplex editing
Growth, Selection (11 days total)
D5 Sample (baseline)
D8 Sample
D11 Sample
Endogenous Locus
Essential gene
CRISPR
ACG
Programmed HDR Library
Genome editing in many cells
Endogenous Loci
ACG
T
G
Selective PCR on gDNA
wt
edits
Seq
gDNA haplotype counts
D5
D8
D11
Enrichment Scores
SNV scores
Amino acid scores